

### **Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

### **Listing of Claims:**

1. (Currently amended) A transgenic nonhuman animal comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene, wherein the allele is rendered nonfunctional by deletion of exons 4-8.
2. (Previously presented) The transgenic nonhuman animal of claim 1 that is homozygous for the allele.
3. (Previously presented) The transgenic nonhuman animal of claim 1 that is a rodent.
4. (Previously presented) The transgenic nonhuman animal of claim 1 that is a mouse.
5. (Previously presented) The transgenic animal of claim 1, wherein the animal or an ancestor thereof was produced by homologous recombination between an endogenous allele of the gene and a construct containing a positive selection marker flanked by segments showing sufficient sequence relatedness to the gene for the construct to recombine with the endogenous allele introducing the positive selection marker into the endogenous allele and rendering it nonfunctional.
6. (Previously presented) The transgenic animal of claim 1, wherein the animal or an ancestor thereof was produced by homologous recombination between an endogenous allele of the gene and a construct containing a positive selection marker flanked by segments showing sufficient sequence relatedness to the gene to undergo homologous recombination with it, these segments being flanked by *frt* recombination sites, whereby the construct recombines with the endogenous gene introducing the positive selection marker and *frt* recombination sites into the endogenous allele, and the *frt* recombination

sites undergo recombination with each other thereby excising DNA between the flp recombination sites resulting in a deleted nonfunctional form of the endogenous allele.

7.-8. (Canceled)

9. (Currently amended) The transgenic nonhuman animal of claim [[8]]1, wherein the allele is rendered nonfunctional by homologous recombination with a targeting vector comprising a [[a]] lambda KOS genomic clone of BACE-1.

10.-12 (Canceled)

13. (Previously presented) The transgenic nonhuman animal of claim 1, further comprising a transgene comprising a mutation in the APP gene associated with familial Alzheimer's disease.

14. (Previously presented) The transgenic nonhuman animal of claim 13, wherein the transgene comprises a mutation at codons 595 and 596 of human APP695, or an isoform or fragment thereof, wherein the amino acid residues at positions corresponding to positions 595 and 596 are asparagine and leucine, respectively.

15. (Previously presented) The transgenic nonhuman animal of claim 13, wherein the transgene comprises a mutation at codon 717 of APP770 or an isoform or fragment of APP770 having a mutant amino acid residue at position 717.

16. (Previously presented) The transgenic nonhuman animal of claim 13, wherein the mutant amino acid residue is isoleucine, phenylalanine or glycine.

17. (Previously presented) The transgenic nonhuman animal of claims 13, wherein the animal is homozygous for the non-functional allele.

18. (Previously presented) The transgenic nonhuman animal of claim 13, wherein the animal is heterozygous for the transgene.

19. (Previously presented) A cortical cell culture derived from the transgenic animal of claim 1.
20. (Previously presented) The cortical cell culture of claim 19, wherein the cell culture is a primary cell culture.
21. (Previously presented) The cortical cell culture of claim 19, wherein the cell culture comprises a detectable amount of a peptide recognized by an antibody that recognizes residues 13-28 of A $\beta$ .
22. (Currently amended) A method for screening for an inhibitor of the production by a protease other than beta-secretase ("non-beta-secretase protease") of a peptide recognized by an antibody that recognizes residues 13-28 of A $\beta$ , comprising  
    exposing ~~a the transgenic animal of claim 1~~ a nonhuman animal comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene or a cortical cell culture derived therefrom to an agent, and  
    detecting the peptide produced in the transgenic animal or cell culture exposed to the agent,  
    wherein a reduced amount of peptide produced in the exposed transgenic animal or cell culture relative to a transgenic animal or cell culture which has not been exposed to the agent is indicative of inhibitory activity.
23. (Previously presented) The method of claim 22, wherein a cortical cell culture is exposed to the agent.
24. (Previously presented) The method of claim 22, wherein the cortical cell culture is a primary cell culture.
25. (Currently amended) A method of analyzing potential side-effects for an inhibitor of beta-secretase, comprising:

exposing a transgenic ~~animal of claim 1~~ nonhuman animal comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene or a cortical cell culture derived therefrom to an inhibitor of beta secretase; and measuring whether there is a change in the level of at least one component of the transgenic animal or cortical cell responsive to the administration of the inhibitor; wherein a change in the level of at least one component indicates a potential side effect.

26. (Previously presented) The method of claim 25, wherein the measuring step measures changes in the levels of a plurality of mRNA species.
27. (Currently amended) An embryonic ~~stem~~ stem cell comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene, wherein the allele is rendered nonfunctional by deletion of exons 4-8.
28. (Previously presented) The embryonic stem cell of claim 27 that is homozygous for the allele.
29. (Previously presented) The embryonic stem cell of claim 27 that is a mouse embryonic stem cell.
30. (Previously presented) The embryonic stem cell of claim 27 produced by homologous recombination with a targeting vector designed in a way that, upon homologous recombination, exons 4 to 8 of the BACE-1 gene are flanked with FLP recombinase target sites (frt sites).
31. (Previously presented) The embryonic stem cell of claim 30, produced by homologous recombination with a targeting vector designed in a way that, with respect to the genomic locus, the 5' region of homology covered 4.5 kb and the 3' region 4.3 kb until the third frt site, and an additional 1.5 kb further 3'.

32. (Currently amended) The embryonic stem cell of claim 27 that is ~~homozygous~~ homozygous for a nonfunctional allele lacking exons 4-8 of BACE-1.
33. (Currently amended) The embryonic stem cell of claim 27, produced by homologous recombination with a first targeting vector that introduces a neomycin resistance gene in the BACE-1 gene and with a second targeting vector that replaces the ~~neomycine~~ neomycin resistance gene with a [[a]] hygromycin resistance gene cassette.
34. (Previously presented) A blastocyst formed by differentiation of an embryonic stem cell as described in claim 27.
35. (New) A method for generating a transgenic nonhuman animal comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene, the method comprising:
- introducing at least one genetic construct into an embryonic stem cell line, the genetic construct comprising a positive selection marker flanked by segments showing sufficient sequence relatedness to the BACE-1 gene to undergo homologous recombination with it, these segments being flanked by flt recombination sites;
  - screening for cells in which recombination has occurred between the genetic construct and the endogenous gene;
  - injecting the embryonic stem cells which have undergone recombination into blastocysts from a nonhuman animal to generate chimeric nonhuman animals;
  - breeding the chimeric nonhuman animals with nonhuman animals of the type which provided the blastocysts to generate the chimeric nonhuman animals to generate nonhuman animals heterozygous for the nonfunctional allele of BACE-1; and
  - breeding the nonhuman animals heterozygous for the nonfunctional allele of BACE-1 with nonhuman animals transgenic for flp recombinase resulting in a nonfunctional form of the endogenous BACE-1 allele.

36. (New) The method of claim 35, wherein the allele is rendered nonfunctional by deletion of at least a segment from exon 1.
37. (New) The method of claim 35, wherein the allele is rendered nonfunctional by deletion of exons 4-8.
38. (New) A transgenic nonhuman animal comprising at least one nonfunctional allele of a beta-secretase-1 (BACE-1) gene, wherein the animal or an ancestor thereof was produced by homologous recombination between an endogenous allele of the gene and a construct containing a positive selection marker flanked by segments showing sufficient sequence relatedness to the gene to undergo homologous recombination with it, these segments being flanked by *frt* recombination sites, whereby the construct recombines with the endogenous gene introducing the positive selection marker and *frt* recombination sites into the endogenous allele, and the *frt* recombination sites undergo recombination with each other thereby excising DNA between the *flp* recombination sites resulting in a deleted nonfunctional form of the endogenous allele.
39. (New) The transgenic nonhuman animal of claim 38 that is homozygous for the allele.
40. (New) The transgenic nonhuman animal of claim 38 that is a rodent.
41. (New) The transgenic nonhuman animal of claim 38 that is a mouse.
42. (New) The transgenic nonhuman animal of claim 38, wherein the allele is rendered nonfunctional by deletion of at least a segment of an exon of the gene.
43. (New) The transgenic nonhuman animal of claim 38, wherein the allele is rendered nonfunctional by deletion of at least a segment from exon 1.
44. (New) The transgenic nonhuman animal of claim 38, wherein the allele is rendered nonfunctional by a 165 base pair deletion of exon 1 starting from 2 basepairs past the initiating methionine and extending through the end of exon 1 replaced with an

expression cassette in the targeting vector electroporated into 129 ES cells to generate the transgenic nonhuman animal.

45. (New) The transgenic nonhuman animal of claim 38, wherein the allele is rendered nonfunctional by deletion of exons 4-8.
46. (New) The transgenic nonhuman animal of claim 38, further comprising a transgene comprising a mutation in the APP gene associated with familial Alzheimer's disease.
47. (New) The transgenic nonhuman animal of claim 46, wherein the transgene comprises a mutation at codons 595 and 596 of human APP695, or an isoform or fragment thereof, wherein the amino acid residues at positions corresponding to positions 595 and 596 are asparagine and leucine, respectively.
48. (New) The transgenic nonhuman animal of claim 46, wherein the transgene comprises a mutation at codon 717 of APP770 or an isoform or fragment of APP770 having a mutant amino acid residue at position 717.
49. (New) The transgenic nonhuman animal of claim 46, wherein the mutant amino acid residue is isoleucine, phenylalanine or glycine.
50. (New) A cortical cell culture derived from the transgenic animal of claim 38.
51. (New) The cortical cell culture of claim 38, wherein the cell culture is a primary cell culture.
52. (New) The cortical cell culture of claim 38, wherein the cell culture comprises a detectable amount of a peptide recognized by an antibody that recognizes residues 13-28 of A $\beta$ .